



US009428794B2

(12) **United States Patent**
Farmer et al.(10) **Patent No.:** **US 9,428,794 B2**(45) **Date of Patent:** **Aug. 30, 2016**(54) **METHODS OF DEPLETING A TARGET NUCLEIC ACID IN A SAMPLE AND KITS FOR PRACTICING THE SAME**(71) Applicant: **Clontech Laboratories, Inc.**, Mountain View, CA (US)(72) Inventors: **Andrew Farmer**, Mountain View, CA (US); **Ian Oglesby**, Mountain View, CA (US); **Magnolia Bostick**, Mountain View, CA (US); **Steve Oh**, Mountain View, CA (US)(73) Assignee: **Takara Bio USA, Inc.**, Mountain View, CA (US)

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(21) Appl. No.: **14/023,354**(22) Filed: **Sep. 10, 2013**(65) **Prior Publication Data**

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Related U.S. Application Data

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(51) **Int. Cl.**
C12Q 1/68 (2006.01)(52) **U.S. Cl.**
CPC **C12Q 1/6806** (2013.01); **C12Q 1/6848** (2013.01)(58) **Field of Classification Search**
None
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

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Primary Examiner — Aaron Priest(74) *Attorney, Agent, or Firm* — Otto C. Guedelhoefer, IV; Bret E. Field; Bozicevic, Field & Francis LLP(57) **ABSTRACT**

Provided are methods of depleting a target nucleic acid in a sample. The methods include contacting a target nucleic acid with two or more polymers that specifically hybridize to the target nucleic acid, and cleaving the hybridized regions of the target nucleic acid to deplete the target nucleic acid in the sample. Kits for practicing the subject methods are also provided.

22 Claims, 11 Drawing Sheets

FIG. 1

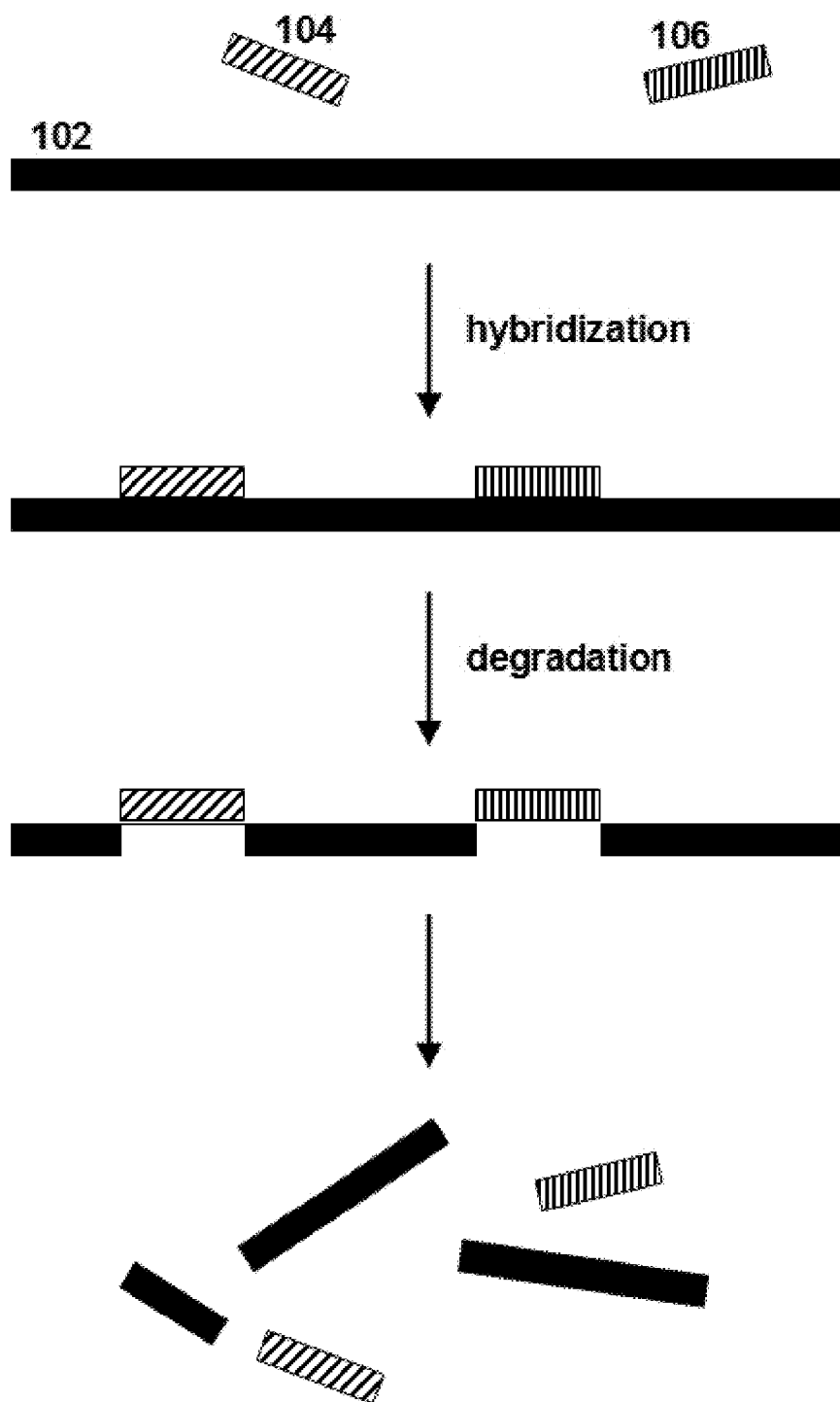


FIG. 2

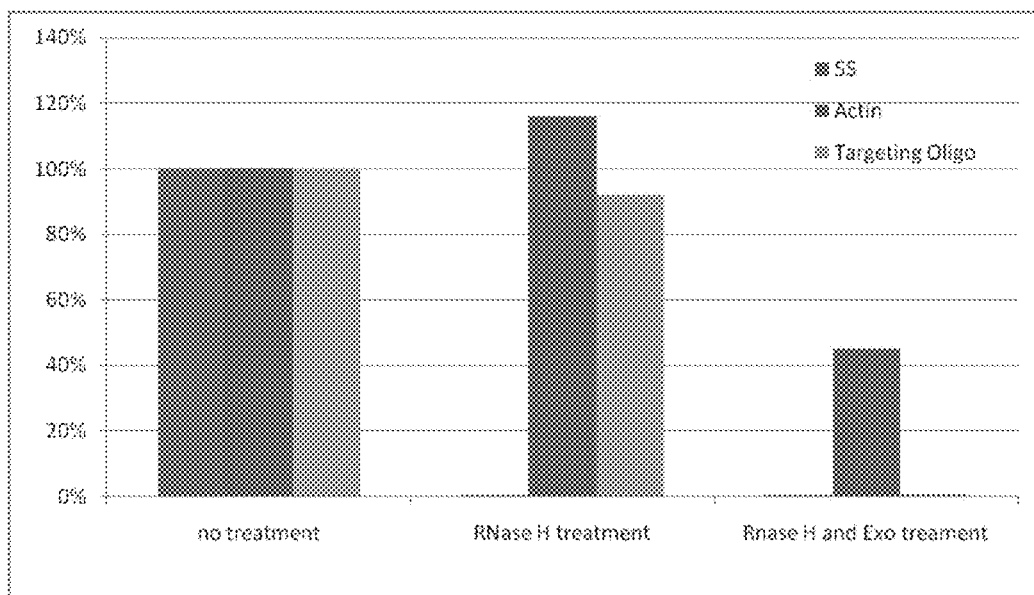


FIG. 3

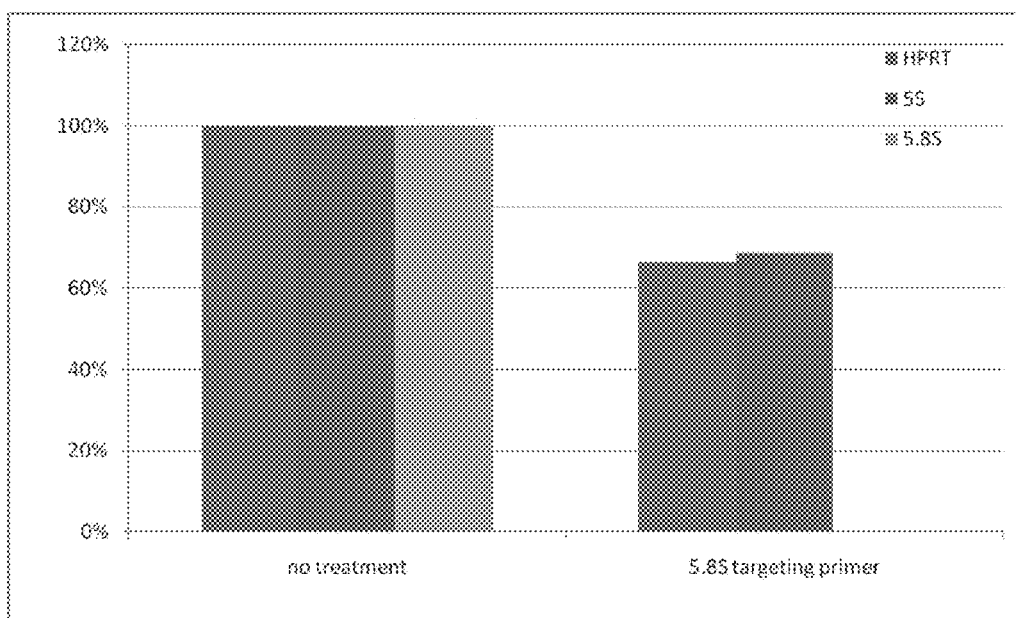


FIG. 4

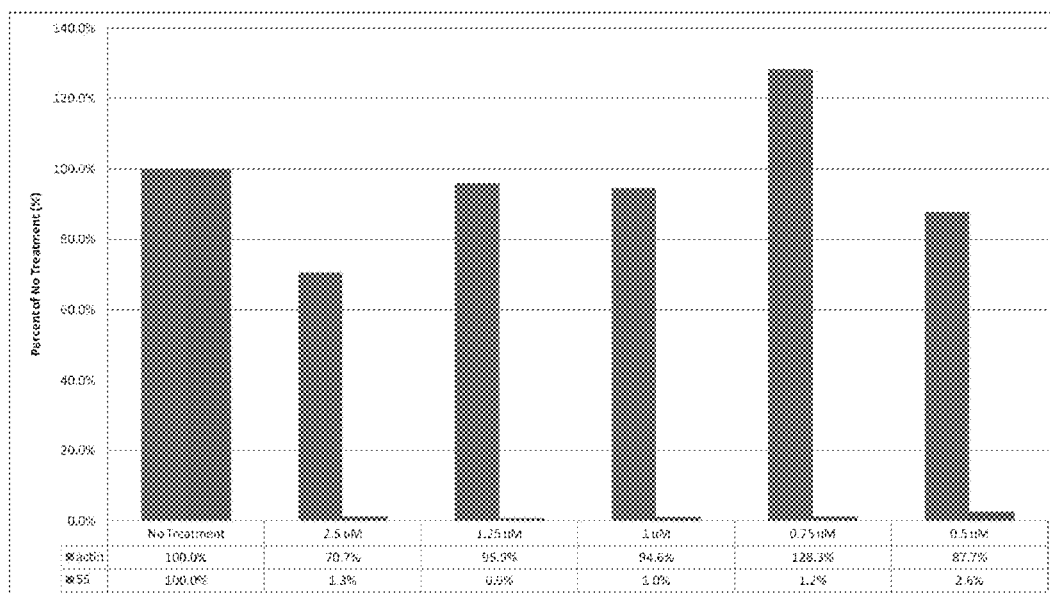


FIG. 5

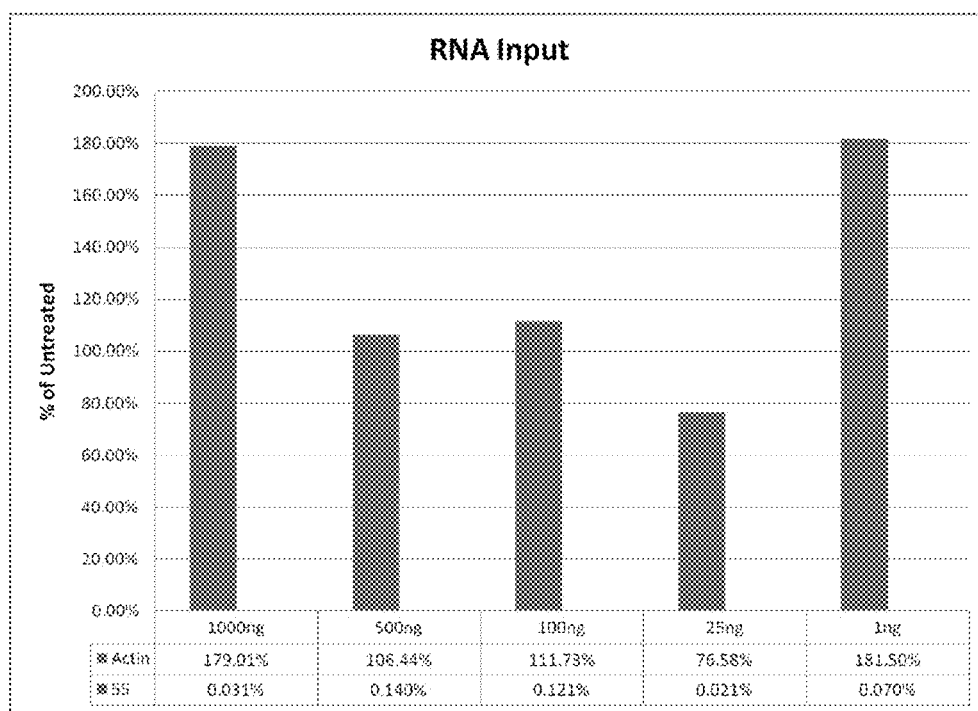


FIG. 6

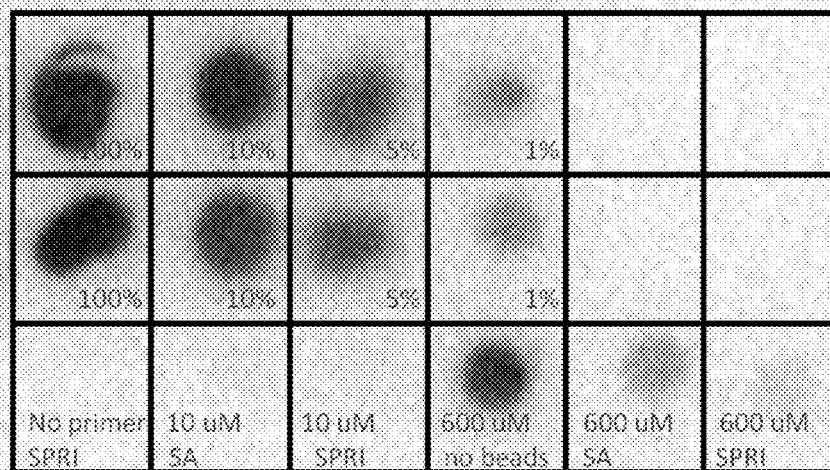


FIG. 7

5s1	/5Biosg/ggtattcccagcggtctcccatccaagta (SEQ ID NO:01)
5.8s1	/5Biosg/cacgagccgagtgatccaccgctaagagtc (SEQ ID NO:02)
5.8s2	/5Biosg/tcacattaattctcgagctagctcggttc (SEQ ID NO:03)
5.8s3	/5Biosg/aagtgcgttcgaagtgatgatcaatgt (SEQ ID NO:04)
5.8s4	/5Biosg/gttcctccggggtacgcctgtctgagcg (SEQ ID NO:05)
18s1	/5Biosg/gcatatgctactggcaggatcaaccaggta (SEQ ID NO:06)
18s2	/5Biosg/cgtgcgtactcagacatgcatggcttaatc (SEQ ID NO:07)
18s3	/5Biosg/aactgatitaaatgagccattcgagtttca (SEQ ID NO:08)
18s4	/5Biosg/ttatccaagtaggagaggagcgagcgacca (SEQ ID NO:09)
18s5	/5Biosg/cagcgcccgctggcatgtattagctctag (SEQ ID NO:10)
18s6	/5Biosg/tgatctgataaatgcacgcaccccccg (SEQ ID NO:11)
18s7	/5Biosg/GCCGGGGCCGGAGAGGGGCTGACCGGGTTG (SEQ ID NO:12)
18s8	/5Biosg/gcccgaggttatctagagtcaccaaagccg (SEQ ID NO:13)
18s9	/5Biosg/gttcgaatgggtcgctcgccgcacggggg (SEQ ID NO:14)
18s10	/5Biosg/gtaggcacggcgactaccatcgaaagttga (SEQ ID NO:15)
18s11	/5Biosg/ggaatgaaccctgattccccgtcacccgt (SEQ ID NO:16)
18s12	/5Biosg/cttccttgatgtggtagccgtttctcagg (SEQ ID NO:17)
18s13	/5Biosg/cctccccgggtcgggagtggttaatttcg (SEQ ID NO:18)
18s14	/5Biosg/gggcctcgaagagtcctgtattgttattt (SEQ ID NO:19)
18s15	/5Biosg/tcctcgttaaaggatttaaagtgactcat (SEQ ID NO:20)
18s16	/5Biosg/AGCTGGAATTACCGCGCTGCTGGCACCAG (SEQ ID NO:21)
18s17	/5Biosg/tttaactgcagcaacttaataacgctat (SEQ ID NO:22)
18s18	/5Biosg/gcggaccgcccgcctcccaagatccaa (SEQ ID NO:23)
18s19	/5Biosg/gagaggcaagggcggggacggcggtggc (SEQ ID NO:24)
18s20	/5Biosg/gccccgggacactcagctaagagcatcg (SEQ ID NO:25)
18s21	/5Biosg/tgcttgaacactctaatttttcaaagta (SEQ ID NO:26)
18s22	/5Biosg/ttccattattctagctgcggtatccaggc (SEQ ID NO:27)
18s23	/5Biosg/cctcagttccgaaaaccaaaaatagaac (SEQ ID NO:28)
18s24	/5Biosg/gcgcaatacgaatgccccggcgtccctc (SEQ ID NO:29)
18s25	/5Biosg/tggtcgctcttgccgggtccaagaatttc (SEQ ID NO:30)
18s26	/5Biosg/TAATGAAAACATTCTTGGCAAATGCTTTCG (SEQ ID NO:31)
18s27	/5Biosg/tacgacggtatctgatcgtcttgaacctc (SEQ ID NO:32)
18s28	/5Biosg/tacgacggtatctgatcgtcttgaacctc (SEQ ID NO:33)
18s29	/5Biosg/cgcccgcgcacgcggcgtcgcatgttta (SEQ ID NO:34)
18s30	/5Biosg/TTTGCAACCATACTCCCCCGGAACCCAAA (SEQ ID NO:35)
18s31	/5Biosg/gctccactctggtggtgcccttcgctcaa (SEQ ID NO:36)
18s32	/5Biosg/ccgggtgaggtttcccggttgagtcaaat (SEQ ID NO:37)
18s33	/5Biosg/agaaagagctatcaatctgtcaatcctgtc (SEQ ID NO:38)

FIG. 7 (cont'd)

18s34	/5Biosg/accaactaagaacggccatgcaccaccacc(SEQ ID NO:39)
18s35	/5Biosg/agtctcgttcgttatcggaattaaccagac(SEQ ID NO:40)
18s36	/5Biosg/gccgaccgctcgggggtcgcgtaactagtt(SEQ ID NO:41)
18s37	/5Biosg/gtggctgaacgccacttgtccctctaagaa(SEQ ID NO:42)
18s38	/5Biosg/catctaaggcatcacagacctgtattgc(SEQ ID NO:43)
18s39	/5Biosg/cacgctgagccagtcagtgtagcgcgctg(SEQ ID NO:44)
18s40	/5Biosg/gttcaacgggttaccgcgcctgccgctg(SEQ ID NO:45)
18s41	/5Biosg/tggggaataattgcaatccccgatccccatt(SEQ ID NO:46)
18s42	/5Biosg/gcaagcttatgacccgcacttactgggaat(SEQ ID NO:47)
18s43	/5Biosg/gcgacggcggtgtgtacaaagggcaggga(SEQ ID NO:48)
18s44	/5Biosg/gccgatccgagggcctcactaaaccatcca(SEQ ID NO:49)
18s45	/5Biosg/tctcagcgctccgccaggccgtggccga(SEQ ID NO:50)
18s46	/5Biosg/CCTTGTTACGACTTTTACTTCTCTAGATA(SEQ ID NO:51)
18s47	/5Biosg/taatgatccttcgcaggttcacctacgg(SEQ ID NO:52)
28s1	/5Biosg/gcgggtcgccacgtctgatctgaggtcgcg(SEQ ID NO:53)
28s2	/5Biosg/ttagtttcttctcctcgctgactaatatg(SEQ ID NO:54)
28s3	/5Biosg/gctcttcctgttcactcgcgttactgag(SEQ ID NO:55)
28s4	/5Biosg/GCGCCCGCGCGGGGCGGGGATTCGCGCGC(SEQ ID NO:56)
28s5	/5Biosg/cccacgagcggcgccgggagcgggtcttc(SEQ ID NO:57)
28s6	/5Biosg/ccgtccacgggctgggctcgcagagaagg(SEQ ID NO:58)
28s7	/5Biosg/CCCGGCGCGCGGGGCGGCTACCGGCCTC(SEQ ID NO:59)
28s8	/5Biosg/cgcttgggctgcattccaagcaaccga(SEQ ID NO:60)
28s9	/5Biosg/gtctcgtgccgtatttagccttagatgga(SEQ ID NO:61)
28s10	/5Biosg/TCAACTTCCCTTACGGTACTTGTGACTA(SEQ ID NO:61)
28s11	/5Biosg/ctcttaacggtttcacgcccttgaactc(SEQ ID NO:63)
28s12	/5Biosg/tgaatcctcgggaggactgcgggacccc(SEQ ID NO:64)
28s13	/5Biosg/GGGCCGCCGACACGGCCGGACCCGCCGCCG(SEQ ID NO:65)
28s14	/5Biosg/gggcggglggagggtcggagggaacgggg(SEQ ID NO:66)
28s15	/5Biosg/AGCCCGCCCTCCGGGGAGGAGGAGGAGG(SEQ ID NO:67)
28s16	/5Biosg/ccggcccccgcacccccgcacccgc(SEQ ID NO:68)
28s17	/5Biosg/cggtcgcgggtcggggacgggtccccgcc(SEQ ID NO:69)
28s18	/5Biosg/cgcggcgccacgcgcggtggaatgcgcc(SEQ ID NO:70)
28s19	/5Biosg/accttccccgcgggccttccagcgtcc(SEQ ID NO:71)
28s20	/5Biosg/CGGGGGAGGAGGAGGACGGACGGACGGACG(SEQ ID NO:72)
28s21	/5Biosg/CGCCCTCCCGAGGAGGACGCGGGCCGGG(SEQ ID NO:73)
28s22	/5Biosg/ctcgggggggtttcgggtccgcgcgcc(SEQ ID NO:74)
28s23	/5Biosg/ccgggattcggcgagtgtgtcgcggggg(SEQ ID NO:75)
28s24	/5Biosg/GAGAGCGCGGCGACGGGTCTCGCTCCCTCG(SEQ ID NO:76)
28s25	/5Biosg/acccccctcgggggattccccgcggggg(SEQ ID NO:77)
28s26	/5Biosg/ccccccacgaggagacgcggcgcgcccc(SEQ ID NO:78)
28s27	/5Biosg/ggggtgggagagcggtcgcgcgtgggagg(SEQ ID NO:79)
28s28	/5Biosg/CCGCGCGCGGCACCCCCCGTCGCGGGG(SEQ ID NO:80)

FIG. 7 (cont'd)

28s29	/5Biosg/gcgcaactggggacagtcgcccccccc(SAQ ID NO:81)
28s30	/5Biosg/agagaacctccccgggcccagcgcgga(SAQ ID NO:82)
28s31	/5Biosg/tccgcccgtccccctcttcggggagcgcg(SAQ ID NO:83)
28s32	/5Biosg/ggggtcggcgcgacgtcggctaccaccc(SAQ ID NO:84)
28s33	/5Biosg/gcacgtgttagactccttggtccgtgttc(SAQ ID NO:85)
28s34	/5Biosg/cattgcgccacggcggtttcgtgcagcc(SAQ ID NO:86)
28s35	/5Biosg/ggatcccaactcggccggcgagcgccgg(SAQ ID NO:87)
28s36	/5Biosg/GTGGTGGCGCCCTCGGCGGACTGGAGAGGCC(SAQ ID NO:88)
28s37	/5Biosg/cgtgcgctcgtctccacccccggcg(SAQ ID NO:89)
28s38	/5Biosg/ccctgccagcatagttcaccatcttcg(SAQ ID NO:90)
28s39	/5Biosg/ggaccgctacggacctccaccagatttc(SAQ ID NO:91)
28s40	/5Biosg/tgccccataccaggtcggagaccgat(SAQ ID NO:92)
28s41	/5Biosg/cttcggagggaaccagctactagatggttc(SAQ ID NO:93)
28s42	/5Biosg/gatagctggcgctctcgagaccgacga(SAQ ID NO:94)
28s43	/5Biosg/ctaatactcgtttaccggataaaactgc(SAQ ID NO:95)
28s44	/5Biosg/gccgaacgatctcaacctatttcaact(SAQ ID NO:96)
28s45	/5Biosg/ccggctccacgccagcgagccggcttctt(SAQ ID NO:97)
28s46	/5Biosg/gcttaccaaaagtggccactagcgactcg(SAQ ID NO:98)
28s47	/5Biosg/ccttaaccggcggttcggttcaccccgag(SAQ ID NO:99)
28s48	/5Biosg/aacaccttttctggggtctgatgagcgtcg(SAQ ID NO:100)
28s49	/5Biosg/tccgacttccatggccacgctcgtctgc(SAQ ID NO:101)
28s50	/5Biosg/ttgattcggcaggtgagttgtacacactc(SAQ ID NO:102)
28s51	/5Biosg/CGACGCTCCAGCGCCATCCATTTTCAGGGC(SAQ ID NO:103)
28s52	/5Biosg/gccgctcccgtccactctcgactcggcg(SAQ ID NO:104)
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28s54	/5Biosg/gaaggacccacaccccccgccgccc(SAQ ID NO:106)
28s55	/5Biosg/AGGAGGGGAGGAGGCGTGGGGGGGGGGCG(SAQ ID NO:107)
28s56	/5Biosg/cgtagcgtccggggctccggggcg(SAQ ID NO:108)
28s57	/5Biosg/gcttaagggtcacgcagcgccctcta(SAQ ID NO:109)
28s58	/5Biosg/CCTGCGGCGGCTCCACCCGGCCCGCGCC(SAQ ID NO:110)
28s59	/5Biosg/cttcaaagttctcgtttgaatattgtac(SAQ ID NO:111)
28s60	/5Biosg/gttcaactgctgttcacatggaaccttct(SAQ ID NO:112)
28s61	/5Biosg/ggaacggcgctcggcatctctcaggaccg(SAQ ID NO:113)
28s62	/5Biosg/cgatcggccgagggaacggaggccatcgc(SAQ ID NO:114)
28s63	/5Biosg/ccgccactccggattcggggatctgaacct(SAQ ID NO:115)
28s64	/5Biosg/GTTACCGCACTGGACGCTCGCGGCGCCCA(SAQ ID NO:116)
28s65	/5Biosg/TCTCCCGGGGCTCCCGCGGCTTCTCCGG(SAQ ID NO:117)
28s66	/5Biosg/CCATTCCAGGGCGCCCTGCCCTTCAAAAG(SAQ ID NO:118)
28s67	/5Biosg/ggaaccgagcgtttccaaggcacggcc(SAQ ID NO:119)
28s68	/5Biosg/TCTCCCCGGATTTCAGGGCCAGCGAGA(SAQ ID NO:120)
28s69	/5Biosg/atatgggtacggccggcgagattaca(SAQ ID NO:121)
28s70	/5Biosg/caacatgccagaggctttcaccttgaga(SAQ ID NO:122)
28s71	/5Biosg/tacggatccggttgccgacttccctacc(SAQ ID NO:123)

FIG. 7 (cont'd)

28s72	/5Biosg/cgaccgacccagcccttagagccaatcctt(SEQ ID NO:124)
28s73	/5Biosg/CGCCTCGTCCAGCCGCGCGCGCCAGC(SEQ ID NO:125)
28s74	/5Biosg/ggggggtgccccggggcgtggggggggcgcg(SEQ ID NO:126)
28s75	/5Biosg/GCGCGGGGTGGGGCGGGGAGGGCCGCGAG(SEQ ID NO:127)
28s76	/5Biosg/ggggagagagagagagaggcgcggggc(SEQ ID NO:128)
28s77	/5Biosg/cacgcgggcgtccccggggagggggagg(SEQ ID NO:129)
28s78	/5Biosg/gccccgccccgaccccttctcccccg(SEQ ID NO:130)
28s79	/5Biosg/cgccggcccccggtgccccggccccct(SEQ ID NO:131)
28s80	/5Biosg/AAGGGCCCGCTCGCGTCCAGAGTCGCCGC(SEQ ID NO:132)
28s81	/5Biosg/CCGGGCTCCCCGGGGCGGCCGACGCCC(SEQ ID NO:133)
28s82	/5Biosg/GACGAGACGTGGGGTGGGGGGGGGGCGCG(SEQ ID NO:134)
28s83	/5Biosg/CCGCCGCCGACCGCTCCCGCCCCAGCG(SEQ ID NO:135)
28s84	/5Biosg/GGGGTAGGGCGGGGGACGAACGCCCGC(SEQ ID NO:136)
28s85	/5Biosg/AGGAGGAGGGGGAACGGGGGGCGGACGGG(SEQ ID NO:137)
28s86	/5Biosg/GCCGCGCGCGAGGAGGAGGGGGGAACGGG(SEQ ID NO:138)
28s87	/5Biosg/ggcggacccggcggggggaccggccgcg(SEQ ID NO:139)
28s88	/5Biosg/ccggcgaggcgaggcgccgcgggaaccg(SEQ ID NO:140)
28s89	/5Biosg/ttccctgggtccgaccagttctaagtcgg(SEQ ID NO:141)
28s90	/5Biosg/gcgggccttcgcgatgctttgtttaatta(SEQ ID NO:142)
28s91	/5Biosg/CTGGGAGAAATCACATCGCGTCAACACCC(SEQ ID NO:143)
28s92	/5Biosg/cgtttaccgcgcttcattgaatttcttca(SEQ ID NO:144)
28s93	/5Biosg/TGGCTACCTTAAGAGAGTCATAGTTACTCC(SEQ ID NO:145)
28s94	/5Biosg/tctcgttcattcatgcgcgtactaa(SEQ ID NO:146)
28s95	/5Biosg/ggctgtggtttcgtgtagtagtagga(SEQ ID NO:147)
28s96	/5Biosg/ggtcttcttccccgctgattccgcaagc(SEQ ID NO:148)
28s97	/5Biosg/atgtcttccaccgtccagactagagtca(SEQ ID NO:149)
28s98	/5Biosg/gggggggcgccggggcctccacttattct(SEQ ID NO:150)
28s99	/5Biosg/CGGACCCCGCCCCGGCCCTCGCGGGGAC(SEQ ID NO:151)
28s100	/5Biosg/aaaaacgatcagtagtggtatttcaccg(SEQ ID NO:152)
28s101	/5Biosg/GGGCTCGCCCCCGCCTACCGGGTCAGT(SEQ ID NO:153)
28s102	/5Biosg/CGCGCGCGCGGGCGCTTGGCGCCAGAAGCG(SEQ ID NO:154)
28s103	/5Biosg/aaactccccactggcactglccccggagc(SEQ ID NO:155)
28s104	/5Biosg/gacacctgcgttaccgttgacagggttac(SEQ ID NO:156)
28s105	/5Biosg/gctccacggagggtttctgtcctccctgag(SEQ ID NO:157)
28s106	/5Biosg/tcgtactgaaatcaagatcaagcgagctt(SEQ ID NO:158)
28s107	/5Biosg/ggtcagaaggatcgtgagggcccgctttca(SEQ ID NO:159)
28s108	/5Biosg/tgtggtaacttttctgacacctctgctta(SEQ ID NO:160)
28s109	/5Biosg/cgtcgctatgaacgcttgccgcccacaagc(SEQ ID NO:161)
28s110	/5Biosg/caatgataggaagagccgacatcgaaggat(SEQ ID NO:162)
28s111	/5Biosg/tagtgggtgaacaatccaacgcttggtgaa(SEQ ID NO:163)
28s112	/5Biosg/cctgtctcacgaggtctaaccagctca(SEQ ID NO:164)
28s113	/5Biosg/GGCAACAACATCATCAGTAGGGTAAAC(SEQ ID NO:165)
28s114	/5Biosg/caaatgtctgaacctgcggttcctctcgta(SEQ ID NO:166)

FIG. 7 (cont'd)

28s115	/5Biosg/gtagcttcgccccattggctcctcagccaa(SEQ ID NO:167)
28s116	/5Biosg/gattctgacttagaggcggttcagtcataat(SEQ ID NO:168)
28s117	/5Biosg/cgaggctccgcgggcgctgccgtatcgttcc(SEQ ID NO:169)
28s118	/5Biosg/ccggcggggacaggcgggggaccggctatc(SEQ ID NO:170)
28s119	/5Biosg/tcccgcgcgcggggcgctggagggggg(SEQ ID NO:171)
28s120	/5Biosg/caccggacccgggtcccggcgcgggcggg(SEQ ID NO:172)
28s121	/5Biosg/ctccggccgcgccccgtttccaggacgaa(SEQ ID NO:173)
28s122	/5Biosg/acgaacgtgcgggtgcgtgacgggcgagggg(SEQ ID NO:174)
28s123	/5Biosg/gaagcaggtcgtctacgaatggtttagcgc(SEQ ID NO:175)
28s124	/5Biosg/agcgagggagctgctctgtctacgtacgaaa(SEQ ID NO:176)
28s125	/5Biosg/gacaaacccttggtgtcgagggtgactttc(SEQ ID NO:177)

1

METHODS OF DEPLETING A TARGET NUCLEIC ACID IN A SAMPLE AND KITS FOR PRACTICING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

Pursuant to 35 U.S.C. §119 (e), this application claims priority to the filing date of the U.S. Provisional Patent Application Ser. No. 61/700,727, filed Sep. 13, 2012; the disclosure of which is herein incorporated by reference.

INTRODUCTION

Applications in biomedical research often involve the analysis of specific subsets of nucleic acids present in a complex mixture of other sequences—for example, analysis of gene expression by array hybridization, qPCR or massively parallel sequencing. If the target sequences are known, PCR with specific primer sequences can be used to amplify the desired sequences out of the mixture. In some cases, however, it may be desired to analyze multiple different sequences, perhaps where sequence information is not fully known. Messenger RNAs in eukaryotic systems, for example, may be collectively amplified and analyzed using an oligo-dT primer to initiate first strand cDNA synthesis by priming on the poly A tail, thereby reducing or avoiding contamination by unwanted nucleic acids—such as ribosomal RNAs, mitochondrial RNAs and genomic DNA. A requirement for this approach, however, is that the RNA is intact and not degraded, e.g., the poly A tails are not lost or disconnected from the body of the RNA message. Unfortunately, many otherwise useful and interesting biological specimens—such as biopsied material retained as formalin-fixed and paraffin embedded tissue samples (FFPE samples) often suffer from such degradation making oligo-dT priming impractical for such samples. Further, many interesting RNA sequences do not have poly A tails—e.g., non-coding RNAs and non-eukaryotic RNAs. In such cases, random priming can be used to generally amplify all nucleotide species in the sample. However, random priming will also result in the amplification of potentially unwanted sequences—such as genomic DNA or ribosomal RNA.

SUMMARY

Provided are methods of depleting a target nucleic acid in a sample. The methods include contacting a target nucleic acid with two or more polymers that specifically hybridize to the target nucleic acid, and cleaving the hybridized regions of the target nucleic acid to deplete the target nucleic acid in the sample. Kits for practicing the subject methods are also provided.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 schematically illustrates a method according to one embodiment of the invention.

FIG. 2 shows the graphical results from an experiment in which a targeting primer and RNase H treatment selectively depleted the target RNA and Exonuclease treatment depleted the targeting primer, according to any embodiment of the invention.

FIG. 3 shows the graphical results from an experiment in which a targeting primer and RNase H treatment selectively depleted the target RNA, according to any embodiment of the invention.

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FIG. 4 shows the graphical results from an experiment demonstrating that a targeting primer is capable of selectively depleting 5S transcripts at a range of concentrations, when employed in methods according to an embodiment of the invention.

FIG. 5 shows the graphical results from an experiment demonstrating that the targeting primer is capable of selectively depleting 5S transcripts at a range of total RNA amounts, when employed in methods according to an embodiment of the invention.

FIG. 6 shows the graphical results from an experiment demonstrating that both SPRI and Streptavidin (SA) beads decrease the concentration of biotinylated targeting primer, when employed in methods according to an embodiment of the invention.

FIG. 7 provides a list of primers used in this experiment.

DETAILED DESCRIPTION

Provided are methods of depleting a target nucleic acid in a sample. The methods include contacting a target nucleic acid with two or more polymers that specifically hybridize to the target nucleic acid, and cleaving the hybridized regions of the target nucleic acid to deplete the target nucleic acid in the sample. Kits for practicing the subject methods are also provided.

Before the methods and kits of the present disclosure are described in greater detail, it is to be understood that the methods and kits are not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the methods and kits will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the methods and kits. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the methods and kits, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the methods and kits.

Certain ranges are presented herein with numerical values being preceded by the term “about.” The term “about” is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods belong. Although any methods and kits similar or equivalent to those described herein can also be used in the practice or testing of the methods, representative illustrative methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated

herein by reference to disclose and describe the methods, kits and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present methods and kits are not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

It is noted that, as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

It is appreciated that certain features of the methods and kits, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the methods and kits, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace operable processes and/or devices/systems/kits. In addition, all sub-combinations listed in the embodiments describing such variables are also specifically embraced by the present methods and kits and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present methods and kits. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

In further describing embodiments of the invention, aspects of embodiments of the subject methods will be described first in greater detail. Thereafter, aspects of embodiments of the kits for practicing the subject methods are described in greater detail.

Methods

Embodiments of the present disclosure relate to methods of depleting a target nucleic acid in a sample. The methods include contacting a target nucleic acid with two or more polymers, where each of the two or more polymers specifically hybridizes to distinct regions of the target nucleic acid to generate hybridized regions of the target nucleic acid. The methods further include cleaving the hybridized regions to deplete the target nucleic acid in the sample.

By “depleting a target nucleic acid,” it is meant reducing the percentage of a type of undesired nucleic acid (e.g., ribosomal RNA (rRNA) or one or more particular sub-types thereof) in a sample with respect to the total nucleic acid in the sample. In certain aspects of the present disclosure, after depletion of the target nucleic acid, the percent remaining of the target nucleic acid as compared to the initial amount of target nucleic acid in the sample is 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less, including 0.5%, 0.1%, 0.01%

or less. By depleting a target nucleic acid in a sample, a desired type of nucleic acid (e.g., messenger RNA (mRNA), micro RNA (miRNA), and/or any other desired type of nucleic acid) may be enriched. According to certain embodiments, in a sample in which the target nucleic acid has been depleted, a desired type of nucleic acid is enriched such that the amount of the desired type of nucleic acid relative to the total nucleic acid in the samples increases by 5% or more, such as 10% or more, 25% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, including 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 99.5% or more.

The target nucleic acid is a polymer of any length composed of nucleotides, e.g., ribonucleotides or deoxyribonucleotides, e.g., 10 bases or longer, 20 bases or longer, 50 bases or longer, 100 bases or longer, 500 bases or longer, 1000 bases or longer, 2000 bases or longer, 3000 bases or longer, 4000 bases or longer, 5000 bases or longer, 10,000 bases or longer, 50,000 or longer or more bases.

The nucleic acid targeted for depletion can be any target nucleic acid selected by a practitioner of the subject methods. According to one embodiment, the target nucleic acid is a ribonucleic acid (RNA). The RNA targeted for depletion may be any type of RNA (or sub-type thereof) including, but not limited to, a ribosomal RNA (rRNA), a microRNA (miRNA), a messenger RNA (mRNA), transfer RNA (tRNA), a small nucleolar RNA (snoRNA), a small nuclear RNA (snRNA), a long non-coding RNA (lncRNA), a non-coding RNA (ncRNA), a small interfering RNA (siRNA), a transacting small interfering RNA (ta-siRNA), a natural small interfering RNA (nat-siRNA), a transfer-messenger RNA (tmRNA), a precursor messenger RNA (pre-mRNA), a small Cajal body-specific RNA (scaRNA), a piwi-interacting RNA (piRNA), an endoribonuclease-prepared sRNA (esiRNA), a small temporal RNA (stRNA), a signal recognition RNA, a telomere RNA, a ribozyme, and any combination of RNA types thereof or subtypes thereof. When the target nucleic acid is a RNA, the methods may include depleting all types of RNA in the sample (e.g., ribosomal RNA, transfer RNA, microRNA, and the like), or one or more particular types of RNA. In certain aspects, the target nucleic acid is ribosomal RNA (rRNA). The rRNA may be a eukaryotic 28S, 26S, 25S, 18S, 5.8S, 5S rRNA, or any combination thereof. In other aspects, the rRNA may be a prokaryotic 23S, 16S, 5S rRNA, or any combination thereof. The subject methods find use in depleting RNAs other than ribosomal RNAs. For example, the target nucleic acid may be a messenger RNA (mRNA), e.g., a highly expressed but clinically irrelevant mRNA from a pool of total RNA or mRNA (e.g., a globulin mRNA in a sample of total or polyA⁺ blood RNA). Other types of RNA may be targeted for depletion, including a precursor messenger RNA (pre-mRNA), a micro RNA (miRNA), a transfer RNA (tRNA), a mitochondrial RNA (mtRNA), and any combination thereof. The target RNA may be a RNA from a particular organism, such as bacterial RNA or yeast RNA.

According to certain embodiments, the target nucleic acid is a DNA, e.g., intronic or inter-geneic DNA to enrich a sample for exonic DNA. DNA-based plasmids/vectors such as those used for in vitro transcription may be targeted for depletion, e.g., to enrich a nucleic acid sample for newly transcribed RNA.

The term “sample”, as used herein, relates to a material or mixture of materials, typically, although not necessarily, in liquid form, containing a mixture of desired and undesired nucleic acids. A sample may be derived from any source of

interest. For example, the sample may be a mixture of undesired and desired nucleic acids isolated from a biological sample such as a single cell, a plurality of cells, organ-
elle, a tissue, an organ, or an organism(s) (e.g., bacteria,
yeast, or the like). Approaches, reagents and kits for isolat-
ing DNA or RNA from such sources are known in the art.
For example, kits for isolating total RNA from cells or
tissues of interest—such as the NucleoSpin® RNA kits by
Clontech Laboratories, Inc. (Mountain View, Calif.)—are
commercially available. In certain aspects, the sample is a
mixture of undesired and desired nucleic acids isolated from
a fixed biological sample, e.g., formalin-fixed, paraffin-
embedded (FFPE) tissue. Total RNA from FFPE tissue may
be isolated using commercially available kits—such as the
NucleoSpin® FFPE RNA kits by Clontech Laboratories,
Inc. (Mountain View, Calif.). Kits for isolating polyA⁺
mRNA from total RNA or directly from cells or tissue—
such as the NucleoTrap® mRNA kits by Clontech Labora-
tories, Inc. (Mountain View, Calif.)—are commercially
available. Small RNAs such as miRNAs, siRNAs, shRNAs
and snRNAs may be isolated from cells, tissues or plasma
using commercially available kits—such as the Nucleo-
Spin® miRNA kits by Clontech Laboratories, Inc. (Moun-
tain View, Calif.).

As set forth above, the subject methods include contacting
the target nucleic acid with two or more polymers. The two
or more polymers can be any polymer suitable for facilitat-
ing the cleavage of the target nucleic acid. Suitable polymers
include, but are not limited to, nucleic acids (e.g., DNA
and/or RNA of any length), proteins, and any modified
variants thereof. According to certain embodiments, the two
or more polymers are two or more nucleic acids. For
example, the two or more polymers may be two or more
DNA molecules, two or more RNA molecules, or two or
more nucleic acids that include at least one DNA and at least
one RNA. In certain aspects, the two or more polymers are
independently selected from a DNA (e.g., a DNA oligo-
nucleotide), an RNA (e.g., an RNA oligonucleotide), a
locked nucleic acid (LNA), a peptide nucleic acid (PNA), a
xeno nucleic acid (XNA), and any combination thereof.

In certain aspects, the two or more polymers are labeled.
The label may be any label chosen by a practitioner of the
subject methods. Labels that find use in the subject methods
include, but are not limited to, biotin, streptavidin, avidin, a
fluorescent dye, a chromophore (e.g., a donor chromophore
or acceptor chromophore capable of participating in fluo-
rescence resonance energy transfer (FRET), and/or the like).
In certain aspects, the two or more polymers are nucleic acid
polymers that are incapable of serving as primers in nucleic
acid extension reactions. For example, the nucleic acid
polymers may have an amino group at their 3' ends to
preclude priming of a nucleic acid synthesis reaction from
the polymers.

According to certain embodiments, the two or more
polymers are two or more oligonucleotides. By “oligonucle-
otide” is meant a single-stranded multimer of nucleotides
from 2 to 500 nucleotides, e.g., 2 to 200 nucleotides.
Oligonucleotides may be synthetic or may be made enzy-
matically, and, in some embodiments, are under 10 to 50
nucleotides in length. Oligonucleotides may contain ribo-
nucleotide monomers (i.e., may be oligoribonucleotides or
“RNA oligonucleotides”) or deoxyribonucleotide monomers
(i.e., may be oligodeoxyribonucleotides or “DNA oligo-
nucleotides”). Oligonucleotides may be 10 to 20, 11 to 30,
31 to 40, 41 to 50, 51–60, 61 to 70, 71 to 80, 80 to 100, 100
to 150 or 150 to 200, up to 500 or more nucleotides in length,
for example.

Each of the two or more polymers is provided in the
sample at any final concentration suitable for the desired
extent of target nucleic acid depletion. For example, the final
quantity concentration of each polymer may independently
be, in certain instances, from 100 nM to 10 μ M, such as 250
nM to 5 μ M, including 500 nM to 2.5 μ M.

Each of the two or more polymers may specifically
hybridizes to distinct regions of the target nucleic acid. By
“specifically hybridizes” is meant the ability of a polymer
(e.g., an oligonucleotide) to hybridize to a region of the
target nucleic acid. In some instances, the polymer hybrid-
izes, if at all, to non-target nucleic acids only partially or
weakly and in some instances the targeting nucleic acid does
not hybridize to any nucleic acid other than to the target
nucleic acid. Whether a polymer specifically hybridizes to a
target nucleic acid is determined by such factors as the
degree of complementarity between the polymer and the
target nucleic acid and the temperature at which the hybrid-
ization occurs, which may be informed by the melting
temperature (T_m) of the polymer. The melting temperature
refers to the temperature at which half of the polymer-target
nucleic acid duplexes remain hybridized and half of the
duplexes dissociate into single strands. The T_m of a duplex
may be experimentally determined or predicted using the
following formula $T_m = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41$ (frac-
tion G+C)–(60/N), where N is the chain length and $[\text{Na}^+]$ is
less than 1 M. See Sambrook and Russell (2001; *Molecular*
Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor
Press, Cold Spring Harbor N.Y., Ch. 10). Other more
advanced models that depend on various parameters may
also be used to predict T_m of polymer duplexes may also be
used depending on various hybridization conditions.
Approaches for achieving specific nucleic acid hybridization
may be found in, e.g., Tijssen, *Laboratory Techniques in*
Biochemistry and Molecular Biology-Hybridization with
Nucleic Acid Probes, part I, chapter 2, “Overview of prin-
ciples of hybridization and the strategy of nucleic acid probe
assays,” Elsevier (1993).

The term “complementary” as used herein refers to a
polymer sequence (e.g., a nucleic acid polymer sequence)
that base-pairs by non-covalent bonds to a target nucleic
acid. In the canonical Watson-Crick base pairing, adenine
(A) forms a base pair with thymine (T), as does guanine (G)
with cytosine (C) in DNA. In RNA, thymine is replaced by
uracil (U). As such, A is complementary to T and G is
complementary to C. In RNA, A is complementary to U and
vice versa. Typically, “complementary” refers to a nucleo-
tide sequence that is at least partially complementary. The
term “complementary” may also encompass duplexes that
are fully complementary such that every nucleotide in one
strand is complementary to every nucleotide in the other
strand in corresponding positions. In certain cases, a nucleo-
tide sequence may be partially complementary to a target, in
which not all nucleotide is complementary to every nucleo-
tide in the target nucleic acid in all the corresponding
positions. For example, the polymer may be perfectly (i.e.,
100%) complementary to the target nucleic acid, or the
polymer and the target nucleic acid may share some degree
of complementarity which is less than perfect (e.g., 70%,
75%, 85%, 90%, 95%, 99%). The percent identity of two
nucleotide sequences can be determined by aligning the
sequences for optimal comparison purposes (e.g., gaps can
be introduced in the sequence of a first sequence for optimal
alignment). The nucleotides at corresponding positions are
then compared, and the percent identity between the two
sequences is a function of the number of identical positions
shared by the sequences (i.e., % identity = # of identical

positions/total # of positions×100). When a position in one sequence is occupied by the same nucleotide as the corresponding position in the other sequence, then the molecules are identical at that position. A non-limiting example of such a mathematical algorithm is described in Karlin et al., Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) as described in Altschul et al., Nucleic Acids Res. 25:389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., NBLAST) can be used. In one aspect, parameters for sequence comparison can be set at score=100, wordlength=12, or can be varied (e.g., wordlength=5 or wordlength=20).

Generating polymers for practicing the subject methods may include identifying unique regions of the target nucleic acid and synthesizing nucleic acid polymers (e.g., two or more oligonucleotides) that are complementary (e.g., perfectly complementary) to the unique regions. By "unique region" is meant a nucleic acid sequence within the target nucleic acid that is not present in any other nucleic acids in the sample of interest. In certain aspects, the unique regions are regions in a ribosomal RNA (e.g., a eukaryotic 28S, 26S, 25S, 18S, 5.8S, or 5S rRNA). Sequence analysis (e.g., a BLAST search) may be performed to determine which regions of a target nucleic acid are unique with respect to the sample of interest. Once the unique regions are identified, nucleic acid polymers of sufficient complementarity, length and/or G:C content may be synthesized such that the polymers specifically hybridize to the unique regions.

The hybridization step can be carried out at any suitable temperature and may be selected depending on, e.g., the minimum temperature required for specific hybridization. Suitable hybridization temperatures may range from 35° C. to 45° C., from 45° C. to 50° C., from 50° C. to 60° C., or from 60° C. to 65° C. Also of interest are lower hybridization temperatures, e.g., where the temperature of the solution is rapidly decreased, e.g., to 30° C. or less, such as 25° C. or less, 20° C. or less, 15° C., 10° C. or less, 5° C. or less, e.g., where the solution is heated and then placed on ice. The temperature of the hybridization step may be the same or different as compared to the temperature of the cleaving step. For example, in certain aspects, the hybridized regions are cleaved enzymatically, and specific hybridization of the oligonucleotides to the target nucleic acid occurs at a temperature at which the enzyme is active and capable of cleaving the hybridized regions (e.g., 37° C., 42° C., 72° C., or the like).

By "two or more polymers" is meant any number of polymers (e.g., nucleic acid polymers) greater than one polymer. In certain aspects, the target nucleic acid is contacted with 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 100, 500, or 1000 or more polymers. One practicing the subject methods may select the number of polymers based on the size and number of different target nucleic acids to be depleted (if one desires to deplete more than one target nucleic acid in the sample) and the desired extent of target nucleic acid cleavage—where the number of polymers that hybridize to distinct regions of the target nucleic acid may be increased to increase the extent of cleavage. The polymers may specifically hybridize to one particular target nucleic acid (e.g., a eukaryotic 28S, 18S, 5.8S, or 5S ribosomal RNA), or two or more different target nucleic acids (e.g., any combination of two or more eukaryotic ribosomal RNAs selected from 28S, 18S, 5.8S, and 5S ribosomal RNA). For example, the subject methods can be used to concurrently deplete the 28S and 18S populations of

eukaryotic rRNAs in a sample using a polymer cocktail that includes two or more nucleic acid polymers that specifically hybridize to 28S rRNAs and two or more nucleic acid polymers that specifically hybridize to 18S rRNAs.

In certain embodiments, the target nucleic acid is denatured prior to hybridization of the two or more polymers to the target nucleic acid. The denaturation step may be useful for removing any secondary structure in a target RNA or for separating the two strands of a double-stranded target DNA, thereby ensuring the availability of the distinct regions of the target nucleic acid for hybridization to the polymers. When the method includes a denaturation step, the denaturation may be performed at a temperature and for a duration suitable to denature the target nucleic acid. The denaturation temperature, for example, may be selected according to the length of the target nucleic acid, the GC content of the target nucleic acid (or relevant sub-region thereof), and/or the like. In certain aspects, the methods include denaturing the target nucleic acid at a temperature of from 50° C. to 98° C., from 55° C. to 95° C., from 60° C. to 90° C., or from 70° C. to 80° C., for example. Denaturing, when employed, may also be accomplished by other protocols, e.g., chemical treatment, such as treatment with NaOH.

Upon specific hybridization of the two or more polymers to the distinct regions of the target nucleic acid, the subject methods include cleaving the hybridized regions of the target nucleic acid to deplete the target nucleic acid in the sample. In certain aspects, the cleaving is performed by contacting the hybridized regions with an enzyme. Approaches for enzymatic cleavage of the hybridized regions of the target nucleic acid may depend on the nature of the hybridized region. In certain aspects, the target nucleic acid is an RNA (e.g., a ribosomal RNA), and the two or more polymers are two or more DNAs (e.g., two or more DNA oligonucleotides). In this example, the hybridized regions constitute DNA/RNA duplexes at the distinct regions of the target RNA, and the cleaving may be carried out by contacting the DNA/RNA duplexes with a ribonuclease that specifically cleaves RNA present in a DNA/RNA duplex, such as RNase H. In other aspects, the target nucleic acid is a DNA, and the two or more polymers are two or more RNAs (e.g., two or more RNA oligonucleotides). Here, the hybridized regions constitute DNA/RNA duplexes at the distinct regions of the target DNA, and the cleaving may be carried out by contacting the DNA/RNA duplexes with an enzyme that specifically cleaves DNA present in a DNA/RNA duplex. In other aspects, the target nucleic acid is an RNA and the two or more polymers are RNA polymers. In this aspect, the target RNA of the resulting RNA/RNA duplex may be cleaved using an enzyme that specifically cleaves an RNA in an RNA/RNA duplex, such as RNase V1.

Enzymes that find use in practicing the subject methods are commercially available and include, but are not limited to: RNase H, RNase V, duplex-specific nuclease (DSN), lambda exonuclease, T7 exonuclease, exonuclease III, RecJ_φ, exonuclease I, exonuclease T, exonuclease V, BAL-31 nuclease, mung bean nuclease, DNase I, micrococcal nuclease, T7 endonuclease I, T5 exonuclease, and any combination thereof. Information regarding nucleases and their substrates is found, e.g., in Mishra et al. (Nucleases: Molecular Biology and Applications (ISBN: 978-0-471-39461-7), the disclosure of which is incorporated herein in its entirety for all purposes. Additional nucleases of interest include, but are not limited to: RNase V1 (e.g., for cleaving dsRNA), oligoribonucleases, (e.g., for cleaving oligonucleotides), exoribonuclease II (e.g., for cleaving mature miRNAs), etc.

The cleaving step may produce a fragmented target nucleic acid, the fragments of which may be removed from the sample if desired. In certain embodiments, the number of the polymers used, the length of the polymers and/or the locations of the distinct regions of the target nucleic acid to which the polymers bind are selected such that all or nearly all of the target nucleic acid fragments are sufficiently small to be removed by nucleic acid purification steps such as ethanol or isopropanol precipitation, spin column purification (e.g., using NucleoSpin® Clean-Up columns by Clontech Laboratories, Inc. (Mountain View, Calif.)), or the like.

A method according to one embodiment of the present disclosure is schematically illustrated in FIG. 1. Nucleic acid polymers **104** and **106** specifically hybridize to distinct regions of target nucleic acid **102**. Upon hybridization of the polymers to the target nucleic acid, the hybridized regions of the target nucleic acid are cleaved to deplete the target nucleic acid in the sample.

Methods of the invention may further include removing the two or more polymers from the sample. For example, a strategy for removing target nucleic acid fragments from the sample (e.g., alcohol precipitation, column purification, or the like) may also be useful for concurrently removing the two or more polymers from the sample, where the polymers are suitable (e.g., sufficiently small) to be removed by the purification approach employed. Alternatively, or additionally, the polymers may be degraded after the hybridized regions of the target nucleic acid are cleaved. In certain aspects, after cleaving the hybridized regions, at least one of the two or more polymers are contacted with an enzyme. The enzyme may be an enzyme (e.g., a nuclease) that specifically cleaves that particular type of polymer (e.g., a DNA, an RNA, or the like). For example, when the target nucleic acid is an RNA and two or more DNA polymers are used, the DNA polymers may be cleaved using a nuclease that specifically cleaves DNA (and not RNA), such as exonuclease I, DNase I, a ribozyme, or any combination thereof.

Other approaches for removing the two or more polymers are possible. According to one embodiment, the polymer is a nucleic acid polymer that includes one or more nucleotides that are not normally present in a DNA or RNA molecule, where the nucleotide not normally present marks the polymer for cleavage when the polymers is contacted with a particular enzyme. For example, the triphosphate form of deoxyuridine (dUTP) is present in living organisms as a metabolic intermediate, but it is rarely incorporated into DNA. When dUTP is incorporated into DNA, the resulting deoxyuridine is promptly removed *in vivo*, e.g. by processes involving the enzyme uracil-N-glycosylase (UDG). In certain aspects, the subject methods employ a DNA polymer in which dUTP is incorporated into the polymer. Following the target nucleic acid cleavage step of the subject methods, the uracil-containing polymer may be contacted with UDG, which cleaves the glycosidic bond between the deoxyribose of the DNA sugar-phosphate backbone and the uracil base, effectively degrading the polymer and/or facilitating their subsequent removal by alcohol precipitation, spin column purification, or other suitable removal strategies.

Also of interest in certain embodiments is the use of a physical separation protocol for removing the polymers from the resultant sample in which the target nucleic acid has been degraded. Any convenient physical separation protocol that selectively removes the polymers from the sample may be employed. An example of a physical separation protocol is one that relies on specific binding of the polymers to a solid phase, where the solid phase is then separate from sample, thereby physically separating the

polymers from the sample. For example, the polymers may be tagged, e.g., with biotin, an oligonucleotide tag, etc., and the contacted with a solid phase comprising a specific binding member for the tag, e.g., avidin where the tag is biotin, a complementary oligo where the tag is an oligonucleotide, etc. The solid phase may vary, where solid phases of interest include, but are not limited to: beads, including magnetic beads, an array, etc.

Turning now to more specific applications of the protocols described herein, according to one embodiment of the present disclosure, a method of depleting ribosomal RNA in a total RNA sample is provided. The method includes contacting a target rRNA with two or more DNA polymers (e.g., two or more DNA oligonucleotides) that specifically hybridize to distinct regions of the target rRNA to generate hybridized regions of the target rRNA. In this embodiment, the DNA polymers are synthesized with dUTP instead of dTTP, and accordingly, have dUTP incorporated therein. The method further includes cleaving the hybridized regions by treatment with RNase H, and subsequently cleaving the DNA polymers by treatment with uracil-N-glycosylase (UDG). After cleavage of the target rRNA and DNA polymers, RNAs are optionally synthesized via a polymerase extension reaction in which random oligonucleotides are used as primers.

In certain aspects, a method of depleting ribosomal RNA in a total RNA sample is provided. The method includes contacting a target rRNA with two or more DNA polymers (e.g., two or more DNA oligonucleotides) that specifically hybridize to distinct regions of the target rRNA to generate hybridized regions of the target rRNA. The method further includes cleaving the hybridized regions by treatment with RNase H, and subsequently cleaving the DNA polymers by treatment with exonuclease I. After cleavage of the target rRNA and DNA polymers, RNAs are optionally synthesized via a polymerase extension reaction in which random oligonucleotides are used as primers.

According to one embodiment, a method of depleting ribosomal RNA in a total RNA sample is provided. The method includes contacting a target rRNA with two or more DNA polymers (e.g., two or more DNA oligonucleotides) that specifically hybridize to distinct regions of the target rRNA to generate hybridized regions of the target rRNA. Reverse transcriptase and dNTPs are then added to the sample and cDNA synthesis using the DNA polymers as primers is performed. The cDNA synthesis reaction generates hybrids between the newly-synthesized cDNA and the target rRNA. The regions of the target rRNA that are hybridized to the DNA polymers and newly-synthesized cDNA is then cleaved by treatment with RNase H. The DNA polymers/cDNA is then cleaved by treatment a suitable enzyme, e.g., with DNase, Exol, etc. After cleavage of the target rRNA and DNA polymers/cDNA, RNAs are optionally synthesized via a polymerase extension reaction in which random oligonucleotides are used as primers.

In certain aspects, a method of depleting ribosomal RNA in a total RNA sample is provided. The method includes contacting a target rRNA with two or more oligonucleotides (e.g., DNA oligonucleotides) that specifically hybridize to distinct regions of the target rRNA to generate hybridized regions of the target rRNA. The method further includes cleaving the hybridized regions by treatment with RNase H. Following RNase H treatment, the oligonucleotides are separated from the remaining RNA in the sample by one of two approaches. First, the oligonucleotides may be removed from the sample by ethanol precipitation, by peg precipitation, by SPRI beads, etc. Alternatively, the remaining RNA

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in the sample may be collected by running the sample through an RNA binding column. Once the non-cleaved RNA is separated from the oligonucleotides, RNAs are optionally synthesized via a polymerase extension reaction in which random oligonucleotides are used as primers.

Kits

Also provided by the present disclosure are kits useful for practicing the subject methods. The kits include two or more polymers, where each of the two or more polymers specifically hybridize to distinct regions of a target nucleic acid. The kits further include a nuclease capable of specifically cleaving the distinct regions of the target nucleic acid when the two or more polymers are hybridized to the target nucleic acid. polymers

The two or more polymers included in the kit may be configured to specifically hybridize (e.g., are complementary) to distinct regions of a target RNA or a target DNA. In certain embodiments, the target nucleic acid is an RNA. The RNA may be a ribosomal RNA, a microRNA, a messenger RNA, a precursor messenger RNA, a transfer RNA, or a mitochondrial RNA, and the polymers may be DNA polymers. Such kits may include a nuclease that specifically cleaves RNA present in a DNA/RNA duplex (e.g., RNase H), compatible buffers for carrying out the cleavage reaction, and/or the like. In certain aspects, the target nucleic acid is a ribosomal RNA (e.g., an rRNA selected from a eukaryotic 28S, 26S, 25S, 18S, 5.8S, or 5S rRNA, an rRNA from prokaryotic sources, etc.), and the kits include two or more DNA polymers configured to specifically hybridize to the ribosomal RNA. In other aspects, the target nucleic acid is an RNA and the two or more polymers are RNA polymers. A kit according to this embodiment may include an enzyme that specifically cleaves an RNA present in an RNA/RNA duplex (e.g., RNase V), compatible buffers for carrying out the cleavage reaction, and/or the like. In some embodiments, the target nucleic acid is a DNA, and the kits include two or more RNA polymers configured to specifically hybridize to the DNA. Such kits may include a nuclease that specifically cleaves DNA present in a DNA/RNA duplex, compatible buffers for carrying out the cleavage reaction, and/or the like.

The subject kits may include any of the polymers or other reagents provided above in the description of the subject methods. Reagents, such as nucleases (e.g., exonuclease I, DNase I, a ribozyme, etc.), may be included in the subject kits for removal of the polymers after a cleaving step is carried out. When the kit includes polymers (e.g., DNA oligonucleotides) that include dUTP as one or more of its polymers, the kit may include uracil-N-glycosylase (UDG) to facilitate cleavage and/or removal of the oligonucleotides from the sample following cleavage of the target nucleic acid. The kits may also include reagents for performing precipitation reactions and/or spin purification columns to remove fragmented target nucleic acids and/or the two or more polymers from the sample, e.g., after a cleavage step is carried out. The subject kits may further include physical separation components, e.g., solid supports, such as beads or arrays, etc.

In certain embodiments, the kits include reagents for isolating a nucleic acid sample from a nucleic acid source. The reagents may be suitable for isolating nucleic acid samples from a variety of sources including single cells, cultured cells, tissues, organs, or organisms. The subject kits may include reagents for isolating a nucleic acid sample from a fixed cell, tissue or organ, e.g., formalin-fixed, paraffin-embedded (FFPE) tissue. Such kits may include one

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or more deparaffinization agents, one or more agents suitable to de-crosslink nucleic acids, and/or the like.

Components of the subject kits may be present in separate containers, or multiple components may be present in a single container. For example, the two or more polymers may be provided in separate containers, or may be provided in a single container.

In addition to above-mentioned components, the subject kit may further include instructions for using the components of the kit to practice the subject method. The instructions for practicing the subject method are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, Hard Disk Drive (HDD) etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

Utility

The subject methods and kits find use in a variety of different applications where, e.g., it is desirable to deplete irrelevant and/or undesired nucleic acids from a nucleic acid sample. By depleting the irrelevant and/or undesired nucleic acids, the complexity of the sample is reduced and the sample is enriched for nucleic acids of interest. Reduced complexity and enrichment of nucleic acids of interest may facilitate and/or improve the results of downstream applications such as nucleic acid amplification, nucleic acid sequencing, gene expression analysis (e.g., by array hybridization, quantitative RT-PCR, massively parallel sequencing, etc.), the preparation of pharmaceutical compositions in which a therapeutic nucleic acid of interest is to be included, and any other applications in which reduced sample complexity and enrichment of nucleic acids of interest is beneficial.

As just one example, the subject methods and kits find use in facilitating gene expression analysis in nucleic acid samples derived from a nucleic acid source (e.g., a formalin fixed, paraffin-embedded tissue sample) in which the integrity of nucleic acids of interest (e.g., mRNAs) is often compromised. For example, the poly-A tails of mRNAs in formalin fixed, paraffin-embedded tissue samples are often absent or degraded to an extent that such tails do not support oligo-dT priming. Accordingly, if the method for enriching the nucleic acid sample for mRNAs is amplification by oligo-dT priming, a substantial proportion of the mRNAs present in the initial sample will not be amplified. The subject methods constitute an approach for enriching nucleic acids of interest that are less sensitive to sample degradation, e.g., because the undesired nucleic acids may be degraded in a manner that utilizes internal sequences of the undesired nucleic acids, which are more likely to remain intact in degraded samples as compared to the poly-A tails of mRNAs.

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The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

I. Experimental Methods:

A. Targeting Primers

60 mer for 5S (with M13R sequence)
(SEQ ID NO: 178)
5'-aagtactaaccaggcccgaccctgcttagctt
ccgaggtcatagctgtttcctgtgtga-3'

3 x 30 mers for 5S
(SEQ ID NO: 179)
5'-aagcctacagcaccgggtattcccaggcgg-3'

(SEQ ID NO: 180)
5'-aagtactaaccaggcccgaccctgcttagc-3'

(SEQ ID NO: 181)
5'-agacgagatcgggcgcttcagggtggtat-3'

4 x 20 mer for 5' biotinylated for 5S
(SEQ ID NO: 182)
5'-biotin-aagcctacagcaccgggtat-3'

(SEQ ID NO: 183)
5'-biotin-tctcccatccaagtactaac-3'

(SEQ ID NO: 184)
5'-biotin-cctgcttagcttcgagatc-3'

(SEQ ID NO: 185)
5'-biotin-gggcgcttcagggtggtat-3'

4 x 30 mers for 5.8S
(SEQ ID NO: 186)
5'-cacgagccgagtgatccaccgctaagagtc-3'

(SEQ ID NO: 187)
5'-tcacattaattctcgagctagctgcgttc-3'

(SEQ ID NO: 188)
5'-aagtgcgttcgaagtgtcgatgatcaatgt-3'

(SEQ ID NO: 189)
5'-gttcctccccgggctacgcctgtctgagcg-3'

B. Oligo Annealing

One μ g of RNA was mixed with DTT (final concentration 1 mM), SmartScribe Buffer (final concentration 1 \times), and primer cocktail (final concentration of primers is 10 μ M). Add water until the volume is 18 μ l. Heat solution to 70° C. for 2 minutes, 37° C. for 2 minutes, 25° C. for 2 minutes, then place on ice for 2 minutes.

C. RNase H treatment

Dilute Takara RNase H to 0.6 U/ μ l in water, add 1 μ l to sample, along with 1 μ l of RNase Inhibitor (Takara). If this is the only enzymatic reaction performed, then incubate solution at 37° C. for 90 minutes. If adding an Exo or DNaseI treatment, only incubate for 1 hour.

D. Exol Treatment

After RNase H treatment, add 1 μ l (5 U/ μ l) of Exol (Takara) and incubate at 37° C. for 30 minutes.

E. Heat Inactivation

If heat inactivating the RNaseH and Exol enzymes, add EDTA (final concentration of 5 mM) and incubate at 80° C. for 20 minutes. Transfer to ice and proceed to reverse transcriptase reaction.

F. SPRI Bead Purification

Follow the instructions for the Beckman Coulter Agencourt AMPure XP kit. Elute with 30 μ l of water and proceed to reverse transcriptase reaction.

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G. Streptavidin in Purification

Wash 12.5 μ l of streptavidin magnetic beads (made at Clontech, 300 ng streptavidin/ μ l of beads) with 500 μ l or 1 ml of Tris buffer, pH 7.4. Add sample solution to washed beads and incubate at 4° C. with rotation for 30 minutes. Remove supernatant (about 25 μ l) and proceed to reverse transcriptase reaction.

H. Reverse Transcriptase Reaction

Take 2-3 μ l of sample after heat activation and/or purification and perform a reverse transcriptase reaction with SMARTScribe protocol (Clontech), using a N6 random primer. Proceed to qPCR reactions.

II. Results

A. A Targeting Primer and RNase H Treatment Selectively Depleted the Target RNA and Exonuclease Treatment Depleted the Targeting Primer.

Total mouse RNA was annealed to a single sixty nucleotide primer which hybridized to the middle of the 5S transcript. The results are shown in FIG. 2. Samples were either untreated (left), treated with RNase H alone (middle), or RNase H and Exonuclease I (right). Remaining RNA was heat inactivated, converted to cDNA with random primers, and amplified by qPCR with primers specific for the 5S (blue), Actin (red), or targeting oligo (green). All sample data are presented as a percentage of the amplification by the same primers in the untreated sample (left). Actin was included to represent the effect of the treatment overall mRNA levels.

B. A Targeting Primer and RNase H Treatment Selectively Depleted the Target RNA.

Total human universal RNA was annealed to four, thirty nucleotide primers which hybridized along the length of the 5.8S transcript. The results are shown in FIG. 3. Samples were either untreated (left) or treated with RNase H (right). Remaining RNA was purified with SPRI beads, converted to cDNA with random primers, and amplified by qPCR with primers specific for the HPRT (blue), 5S (red), or 5.8S (green). All sample data are presented as a percentage of the amplification by the same primers in the untreated sample (left). HPRT and 5S were included to represent the specificity of the targeting primer treatment.

C. The Targeting Primer is Capable of Selectively Depleting 5S Transcripts at a Range of Concentrations.

Total mouse RNA was annealed to four, twenty nucleotide, biotinylated primers which hybridized along the length of the 5S transcript. The results are shown in FIG. 4. Samples were either untreated (no primer, left) or treated with a range of targeting primer concentrations (2.5 μ M, 1.25 μ M, 1 μ M, 0.75 μ M, or 0.5 μ M). All samples were then treated with RNase H, and the remaining RNA was separated from the biotinylated targeting primers with streptavidin-coated beads, converted to cDNA with random primers, and amplified by qPCR with primers specific for Actin (blue) or 5S (red). All sample data are presented as a percentage of the amplification by the same primers in the untreated sample (left). Actin was included to represent the effect of the treatment overall mRNA levels.

D. The Targeting Primer is Capable of Selectively Depleting 5S Transcripts at a Range of Total RNA Amounts.

Total mouse RNA at various amounts (1000 ng, 500 ng, 100 ng, 25 ng, and 1 ng) was annealed to three, thirty nucleotide primers at a concentration of 2.5 μ M each which hybridized along the length of the 5S transcript. The results are shown in FIG. 5. All concentrations of RNA had a treated (with primer) and an untreated sample (without primer). All samples were treated with RNase H, and the remaining RNA was purified with SPRI beads, converted to cDNA with

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random primers, and amplified by qPCR with primers specific for Actin (blue) or 5S (red). All concentrations of RNA are presented as a percentage of the amplification by the same primers in the untreated sample (not shown). Actin was included to represent the effect of the treatment overall mRNA levels.

E. Both SPRI and Streptavidin (SA) Beads Decrease the Concentration of Biotinylated Targeting Primer.

Anti-biotin antibodies were used to visual biotinylated primers cross-linked to a membrane. The results are shown in FIG. 6. A standard curve was created in duplicate (rows 1 and 2) by blotting the equivalent of an entire reaction (100%, 10 μ M primers), 10% of a reaction (1 μ M), 5% (500 nM), or 1% (100 nM). Samples from experiments are presented on the last row. These experiments either had no primer (far left), 10 μ M biotinylated primers (second and third box), or 600 μ M (fourth, fifth, and sixth box). RNA was separated from the biotinylated primers with either streptavidin (SA) or SPRI beads and subsequently blotted on the membrane. The fourth box is a 1:30 dilution of an unpurified reaction. The biotinylated primers employed in this experiment are provided in FIG. 7.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this

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invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

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<211> LENGTH: 30
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<400> SEQUENCE: 44

cacgctgagc cagtcagtgt agcgcgctg 30

<210> SEQ ID NO 45
<211> LENGTH: 30
<212> TYPE: DNA
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 45

gttcaacggg ttaccgcgc ctgccggcgt 30

<210> SEQ ID NO 46
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 46

tggggaataa ttgcaatccc cgatcccat 30

<210> SEQ ID NO 47
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 47

gcaagcttat gaccgcact tactgggaat 30

<210> SEQ ID NO 48
<211> LENGTH: 30
<212> TYPE: DNA
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<400> SEQUENCE: 48

gcgacgggcg gtgtgtacaa agggcaggga 30

<210> SEQ ID NO 49
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<400> SEQUENCE: 49

gccgatccga gggcctcact aaaccatcca 30

<210> SEQ ID NO 50
<211> LENGTH: 30
<212> TYPE: DNA
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<400> SEQUENCE: 50

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tctcagcgct cgcgcagggc cgtgggccga 30

<210> SEQ ID NO 51
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<400> SEQUENCE: 51

ccttggtacg acttttactt cctctagata 30

<210> SEQ ID NO 52
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<400> SEQUENCE: 52

taatgatcct tccgcagggt cacctacgg 29

<210> SEQ ID NO 53
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<400> SEQUENCE: 53

gcgggtcgcc acgtctgac tgaggtcgag 30

<210> SEQ ID NO 54
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<400> SEQUENCE: 54

ttagttttctt ctctccgct gactaatatg 30

<210> SEQ ID NO 55
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<400> SEQUENCE: 55

gctcttcctt gttcactcgc cgttactgag 30

<210> SEQ ID NO 56
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<400> SEQUENCE: 56

gcgccccgcc gcggggcggg gattcggcgc 30

<210> SEQ ID NO 57
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 57

cccacgagcg gcgccgggga gcgggtcttc 30

<210> SEQ ID NO 58
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<400> SEQUENCE: 58

ccgtccacgg gctgggcctc gatcagaagg 30

<210> SEQ ID NO 59
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<400> SEQUENCE: 59

cccggcgcgc cggggggccgc taccggcctc 30

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cgctttgggc tgcattccca agcaaccgga 30

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<400> SEQUENCE: 61

gtctcgtgcc ggtatttagc cttagatgga 30

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<400> SEQUENCE: 62

tcaactttcc cttacggtag ttgttgacta 30

<210> SEQ ID NO 63
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<400> SEQUENCE: 63

ctcttaacgg tttcacgcc tcttgaactc 30

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<210> SEQ ID NO 64
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 64

tgaatcctcc gggcggactg cgcggaacccc

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<400> SEQUENCE: 65

gggcccgcga cacggccgga cccgccgcgcg

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<210> SEQ ID NO 66
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<212> TYPE: DNA
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<400> SEQUENCE: 66

gggcgggtgg aggggtcggg aggaacgggg

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<210> SEQ ID NO 67
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<400> SEQUENCE: 67

agcccgcgcc ctccggggag gaggaggagg

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<210> SEQ ID NO 68
<211> LENGTH: 30
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 68

ccggccccgc ccgcccaccc ccgcacccgc

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<210> SEQ ID NO 69
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<400> SEQUENCE: 69

cggtcgccgg tcgggggacg gtcccccgcc

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<210> SEQ ID NO 70
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<212> TYPE: DNA
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<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 70

cgcgggcgac cgccgcggtg gaaatcgccc 30

<210> SEQ ID NO 71
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 71

accttccccg cgggacctc ccagcgtcc 30

<210> SEQ ID NO 72
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 72

cgggggagga ggaggacgga cggacggacg 30

<210> SEQ ID NO 73
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 73

cgccctcccg agggaggacg cggggccggg 30

<210> SEQ ID NO 74
<211> LENGTH: 30
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 74

ctcggggggg gtttcggtcc cgccgccgcc 30

<210> SEQ ID NO 75
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 75

ccgggattcg gcgagtgtg ctgccggggg 30

<210> SEQ ID NO 76
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 76

gagagcgagg cgacgggtct cgctccctcg 30

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<210> SEQ ID NO 77
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 77

acccccctcg cgggggattc cccgcggggg 30

<210> SEQ ID NO 78
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 78

ccccccacg aggagacgcc ggcgcgcccc 30

<210> SEQ ID NO 79
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 79

ggggtgggag agcggtcgcg ccgtgggagg 30

<210> SEQ ID NO 80
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 80

ccgcgcgcgg ccccccccc gtcgccgggg 30

<210> SEQ ID NO 81
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 81

gcgcactggg gacagtccgc cccgcccccc 30

<210> SEQ ID NO 82
<211> LENGTH: 30
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 82

agagaacctc ccccgggccc gacggcgcca 30

<210> SEQ ID NO 83
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

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<400> SEQUENCE: 83

tccgcgcgtcc ccctcttcgg gggacgcgcg

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<210> SEQ ID NO 84

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 84

gggggtcggcg gcgacgtcgg ctaccacccc

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<210> SEQ ID NO 85

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 85

gcacgtgtta gactccttgg tccgtgttcc

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<210> SEQ ID NO 86

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 86

cattgcgcca cggcggcttt cgtgcgagcc

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<210> SEQ ID NO 87

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 87

ggatcccacc tcggccggcg agcgcgccgg

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<210> SEQ ID NO 88

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 88

gtggtgcgcc ctccggcgac tggagaggcc

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<210> SEQ ID NO 89

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 89

cgtgcgctcg tgetccacct ccccggcgcg

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<210> SEQ ID NO 90

<211> LENGTH: 30

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 90

ccctgcccag gcatagttca ccattcttcg 30

<210> SEQ ID NO 91
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 91

ggaccgctac ggacctccac cagagtttcc 30

<210> SEQ ID NO 92
<211> LENGTH: 30
<212> TYPE: DNA
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 92

tcgcccctat acccaggtcg gacgaccgat 30

<210> SEQ ID NO 93
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 93

cttcggaggg aaccagctac tagatgggtc 30

<210> SEQ ID NO 94
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 94

gatatctggc gctctcgcag acccgacgca 30

<210> SEQ ID NO 95
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 95

ctaatcattc gctttaccgg ataaaactgc 30

<210> SEQ ID NO 96
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 96

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gccgaaacga tctcaaccta ttctcaaact 30

<210> SEQ ID NO 97
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 97

ccggctccac gccagcgagc cgggcttctt 30

<210> SEQ ID NO 98
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 98

gcttaccaaaa agtggccccc taggcactcg 30

<210> SEQ ID NO 99
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 99

ccttaaccgc gcgttcggtt catccgcgag 30

<210> SEQ ID NO 100
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 100

aacacctttt ctggggtctg atgagcgctc 30

<210> SEQ ID NO 101
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 101

tccgacttcc atggccaccg tctgctgtc 30

<210> SEQ ID NO 102
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 102

ttgattcggc aggtgagttg ttacacactc 30

<210> SEQ ID NO 103
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 103

cgacgctcca gcgccatcca ttttcagggc 30

<210> SEQ ID NO 104
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 104

gccgctcccg tccactctcg actgccggcg 30

<210> SEQ ID NO 105
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 105

tccgacgcac accacacgcg cgcgcgcgcg 30

<210> SEQ ID NO 106
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 106

gaaggacccc acacccccgc cgccgcggcc 30

<210> SEQ ID NO 107
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 107

aggaggggag gaggcgtggg ggggggggcg 30

<210> SEQ ID NO 108
<211> LENGTH: 30
<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 108

cgtacgctcc gcggggctcc gggggcgggg 30

<210> SEQ ID NO 109
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 109

gcttcaaggc tcaccgcagc gggcctccta 30

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<210> SEQ ID NO 110
<211> LENGTH: 30
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 110

cctgcggcgg ctccaccgg gccgcgccc 30

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 111

cttcaaagtt ctggttgaa tatttgctac 30

<210> SEQ ID NO 112
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 112

gttcaactgc tgttcacatg gaacccttct 30

<210> SEQ ID NO 113
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 113

ggaacggcgc tcgccatct ctcaggaccg 30

<210> SEQ ID NO 114
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 114

cgatcggccg agggcaacgg aggccatcgc 30

<210> SEQ ID NO 115
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 115

ccgccactcc ggattcgggg atctgaaccc 30

<210> SEQ ID NO 116
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

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<400> SEQUENCE: 116

gttacgcgac tggacgcctc gcggcgccca 30

<210> SEQ ID NO 117

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 117

tctccccggg gctcccgccg gcttctccgg 30

<210> SEQ ID NO 118

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 118

ccattccagg gcgccctgcc cttcacaag 30

<210> SEQ ID NO 119

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 119

ggaaccgcga cgctttccaa ggcacgggcc 30

<210> SEQ ID NO 120

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 120

tctccccggg attttcaagg gccagcgaga 30

<210> SEQ ID NO 121

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 121

atatgggtac ggcccggcgc gagatttaca 30

<210> SEQ ID NO 122

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 122

caacatgccg gaggtgttc accttgagaga 30

<210> SEQ ID NO 123

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<211> LENGTH: 30
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 123

tacggatccg gcttgccgac ttcccttacc 30

<210> SEQ ID NO 124
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 124

cgaccgaccc agcccttaga gccaatcctt 30

<210> SEQ ID NO 125
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 125

cgccctcgtec agccgcggcg cgcgcccagc 30

<210> SEQ ID NO 126
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 126

gggggtgccc cgggcgtggg gggggcggcg 30

<210> SEQ ID NO 127
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 127

gcgcggggtg gggcggggga gggccgcgag 30

<210> SEQ ID NO 128
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 128

ggggagagag agagagagag ggcgcggggc 30

<210> SEQ ID NO 129
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 129

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cacgcggcgc tccccgggg aggggggagg 30

<210> SEQ ID NO 130
<211> LENGTH: 30
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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 130

gccccgcgcg ccccgaccct tctcccccg 30

<210> SEQ ID NO 131
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 131

cgccggcccc cgggtgccc ggccccccct 30

<210> SEQ ID NO 132
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<210> SEQ ID NO 136
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<400> SEQUENCE: 138

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<400> SEQUENCE: 139

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<400> SEQUENCE: 156

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<400> SEQUENCE: 157

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<400> SEQUENCE: 158

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<210> SEQ ID NO 162
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<400> SEQUENCE: 162

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<212> TYPE: DNA

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<220> FEATURE:

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<400> SEQUENCE: 165

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<212> TYPE: DNA

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<210> SEQ ID NO 169

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<210> SEQ ID NO 182
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<220> FEATURE:
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<400> SEQUENCE: 185

gggcgcgttc aggggtggtat 20

<210> SEQ ID NO 186
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<223> OTHER INFORMATION: Synthetic Nucleic Acid Sequence

<400> SEQUENCE: 188

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<210> SEQ ID NO 189
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 <213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 189

gttcctcccg gggetacgcc tgtctgagcg

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What is claimed is:

1. A method of depleting an undesired target ribosomal
 ribonucleic acid (rRNA) in a sample and enriching for a
 desired RNA, the method comprising:

obtaining a total RNA sample comprising a desired RNA
 and an undesired target rRNA;

contacting the sample with a polymer cocktail comprising
 at least two synthetic oligonucleotides selected from
 SEQ ID NOS:06-177 that specifically hybridize to
 distinct regions of an undesired 18S target rRNA or an
 undesired 28S target rRNA in the sample to generate
 hybridized regions of the undesired target rRNA that
 are separated by a gap;

cleaving the hybridized regions to deplete the undesired
 target rRNA in the sample; and

collecting the desired RNA or removing the cleaved
 undesired target rRNA to enrich the for the desired
 RNA.

2. The method according to claim 1, wherein the total
 RNA sample is isolated from a biological sample.

3. The method according to claim 2, wherein the biologi-
 cal sample is selected from the group consisting of: a tissue
 sample, a cell sample, and a bacterial sample.

4. The method according to claim 3, wherein the biologi-
 cal sample is a fixed sample.

5. The method according to claim 1, wherein cleaving the
 hybridized regions comprises contacting the hybridized
 regions with an enzyme.

6. The method according to claim 5, wherein cleaving the
 hybridized regions comprises contacting the hybridized
 regions with a ribonuclease.

7. The method according to claim 6, wherein the ribonu-
 clease specifically cleaves RNA present in an RNA/DNA
 duplex.

8. The method according to claim 6, wherein the ribonu-
 clease is RNase H.

9. The method according to claim 1, wherein, after
 cleaving the hybridized regions, the method comprises
 removing the at least two synthetic oligonucleotides.

10. The method according to claim 9, wherein the at least
 two synthetic oligonucleotides are removed by contacting
 the at least two synthetic oligonucleotides with an enzyme.

11. The method according to claim 10, wherein the at least
 two synthetic oligonucleotides comprise deoxyuridine
 triphosphate (dUTP).

12. The method according to claim 11, wherein the
 method comprises contacting the at least two synthetic
 oligonucleotides with uracil-N-glycosylase (UDG).

13. The method according to claim 9, wherein the at least
 two synthetic oligonucleotides are removed by a separation
 protocol.

14. The method according to claim 13, wherein the
 separation protocol comprises binding the at least two
 synthetic oligonucleotides to a solid support.

15. The method according to claim 1 wherein the unde-
 sired target rRNA comprises 5S rRNA, 5.8S rRNA or a
 combination thereof, the method further comprises:

contacting the sample with a synthetic oligonucleotide
 that specifically hybridizes to distinct regions of an
 undesired target 5S rRNA or an undesired target 5.8S
 rRNA in the sample to generate hybridized regions of
 the undesired target 5S rRNA or the undesired target
 5.8S rRNA; and

cleaving the hybridized regions to deplete the undesired
 target 5S rRNA or the undesired target 5.8S rRNA in
 the sample.

16. The method according to claim 1, further comprising:
 contacting the sample with a synthetic oligonucleotide
 that specifically hybridizes to distinct regions of an
 undesired target mitochondrial ribonucleic acid
 (mtRNA) in the sample to generate hybridized regions
 of the undesired target mtRNA; and

cleaving the hybridized regions to deplete the undesired
 target mtRNA in the sample.

17. The method according to claim 1 further comprising:
 contacting the sample with a synthetic oligonucleotide
 that specifically hybridizes to distinct regions of an
 undesired target transfer RNA (tRNA) in the sample to
 generate hybridized regions of the undesired target
 tRNA; and

cleaving the hybridized regions of the undesired target
 tRNA to deplete the undesired target tRNA in the
 sample.

18. The method according to claim 1, further comprising:
 contacting the sample with a synthetic oligonucleotide
 that specifically hybridizes to distinct regions of an
 undesired target deoxyribonucleic acid (DNA) in the
 sample to generate hybridized regions of the undesired
 target DNA; and

cleaving the hybridized regions of the undesired target
 DNA to deplete the undesired target DNA in the
 sample.

19. The method according to claim 18, wherein cleaving
 the hybridized regions of the undesired target DNA com-
 prises contacting the hybridized regions of the undesired
 target DNA with a deoxyribonuclease.

20. The method according to claim 1, wherein the at least
 two synthetic oligonucleotides of the polymer cocktail com-
 prise sequences that are complementary to unique regions of
 eukaryotic 28S or 18S ribosomal RNA.

21. The method according to claim 1, wherein the at least
 two synthetic oligonucleotides of the polymer cocktail are
 sufficiently small to be removed by a nucleic acid purifica-
 tion step.

22. The method according to claim 21, wherein the at least
 two synthetic oligonucleotides of the polymer cocktail are
 11 to 30 or 31 to 40 nucleotides in length.

* * * * *